Docket No.: 49890(48340)
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Jon A. Weidanz et al.

Application No.: 09/874,907 | Confirmation No.: 3602

Filed: June 5, 2001 | Art Unit: 1644

For: T CELL RECEPTOR FUSIONS AND CONJUGATES AND METHODS OF USE THEREOF | Examiner: R. B. Schwadron

DECLARATION UNDER 37 CFR 1.132

1. I, Hing Wong, declare that I am a resident of the United States. My residential address is 2966 Wentworth, Weston, FL 33332.

2. I am an inventor on the above-identified application (hereafter, "the Application"). I am currently President and CEO of Altor BioScience Corporation, an Assignee of the Application. I earned my Ph.D. in 1980 at the University of Massachusetts. I have published numerous scientific papers in peer-reviewed journals and am an inventor on approximately 100 patents and patent applications.

3. I have reviewed the Office Action dated August 13, 2010 (the "Office Action") in connection with the Application.

4. I have reviewed the Submission Accompanying a Request for Continued Examination filed concurrently herewith.

5. The cited references do not teach either expressly or inherently the subject invention. Additionally, the results of the experiments presented below are unexpected in view of the teachings of the prior art.

6. It is my understanding that the molecules described below (264scTCR/IL-2, c264scTCR/IL-2 and MART-1scTCR/IL-2) are covered by the claims of the Application. Indeed, the 264scTCR/IL-2 fusion molecule is described in detail in the specification. In particular, the construction of the 264scTCR/IL-2 fusion molecule is described in Examples 1-5. The 264scTCR/IL-2 fusion molecule has the following structure: a (TCR-Vα domain)--- (a peptide linker)---(TCR- Vβ domain)---(TCR-Cβ domain fragment)---another peptide linker---(IL-2 fragment). As I understand it, this molecule is covered by the claims of the Application because it is a soluble single-chain T cell receptor ("scTCR") fusion molecule that contains a cytokine (IL-2) connected by a peptide linker to a soluble scTCR; the scTCR has one recognition binding site and the IL-2 has a different recognition binding site; and the scTCR has a Vα domain linked to a Vβ domain by a peptide linker. The c264scTCR/IL-2 and MART-1scTCR/IL-2 fusion proteins also have a similar structure, namely, a (TCR-Vα domain)--- (a peptide linker)---(TCR- Vβ domain)---(TCR-Cβ domain fragment)---another peptide linker---(IL-2 fragment). Therefore, it is my understanding that the c264scTCR/IL-2 and MART-1scTCR/IL-2 fusion molecules are also covered by the claims of the Application patent.

7. The following experiments demonstrate the unexpected efficacy of the molecules claimed in the Application. Based on the publications available at the time this application was filed, it would not have been expected that the claimed molecules would have the enhanced efficacy demonstrated by the experiments described below. This enhanced efficacy make the claimed fusion molecules highly attractive for clinical use.

Enhanced binding of TCR/IL-2 fusion proteins to IL-2R bearing murine and human cells as evidenced by an extended cell surface residency time

The results below indicate that the IL-2 domain of the claimed fusion molecules (c264scTCR/IL-2 and MART-1scTCR/IL-2) exhibit longer cell surface residency time and bind more stably to the IL-2 receptor than does IL2 when not part of the claimed fusion molecules.

This longer residency time and more stable interaction allows the scTCR/IL-2 fusion protein to bind IL-2R-bearing immune cells and more effectively direct the scTCR/IL-2-coated cells against diseased cells expressing the peptide/HLA target, thereby increasing the efficacy of the claimed molecules over that of IL2 alone.

The cell surface residency time of the scTCR/IL-2 fusion protein on the IL-2R-bearing cells influences the ability of the fusion protein to target or bridge effector cells with the TCR-specific tumor cells. To investigate this, binding of the scTCR/IL-2 fusion proteins and recombinant human IL-2 (rhIL-2) to IL-2R bearing CTLL-2 cells was directly compared by flow cytometry. Figure 1 shows the results of such a study in which c264scTCR/IL-2, MART-1scTCR/IL-2 or rhIL-2-coated cells were incubated in media at 37°C for up to 180 min and the level of proteins remaining on the cell surface was detected with anti-IL-2 mAb. As shown at the initial time point (t = 0), addition of equivalent molar amounts of c264scTCR/IL-2, MART-1scTCR/IL-2 or rhIL-2 to CTLL-2 cells resulted in specific staining with PE-labeled anti-IL-2 mAb. Incubation at 37°C resulted in cellular internalization and/or dissociation of the proteins as measured by a decrease in anti-IL-2 mAb binding. The cell surface half-life of rhIL-2 was determined to be 8.2 min by this method, a value similar to the 10 min $t_{1/2}$ reported by Robb et al. (1987. J. Exp. Med. 165:1201). The calculated half-lives of 264scTCR/IL-2 and MART-1scTCR/IL-2 interaction with CTLL-2 IL-2R were approximately 20.3 and 22.7 min, respectively. Similar studies done with activated human PBMC indicated that c264scTCR/IL-2 specifically binds to the human IL-2R bearing cells with a half-life of about 20 min, equivalent to the half-life seen for CTLL-2 cells.

Biological activity of scTCR/IL-2 fusion protein in vitro and in vivo

The results from these studies described below indicate that the c264scTCR/IL-2 and MART-1scTCR/IL-2 fusion molecules showed equivalent IL-2-like biologic activity *in vitro* and *in vivo*.

To demonstrate biological activity of the IL-2 portion of the scTCR/IL-2 fusion protein, IL-2 dependent CTLL-2 cells were cultured with either c264scTCR/IL-2, MART-1scTCR/IL-2 or rhIL-2 at various concentrations and cell viability was assessed using WST-1. For each protein,

a dose-dependent response of CTLL-2 proliferation was observed wherein there was more cell proliferation at higher concentrations of protein. The protein concentration providing a half-maximal effect (EC50) was determined (Fig. 2). Given the four-fold difference in molecular weight between rhIL-2 and the scTCR/IL-2 fusion proteins, the three proteins exhibited similar activity on an equal molar basis (EC50: rhIL-2, 0.15 nM; c264scTCR/IL-2, 0.12 nM; and MART-1scTCR/IL-2, 0.13 nM), indicating that c264scTCR/IL-2 and MART-1scTCR/IL-2 fusion proteins have equivalent bioactivity as rhIL-2 for stimulating immune cell proliferation.

The capability of these proteins to stimulate immune effector cell responses was also examined. Athymic nude mouse splenocytes were incubated for four days in media containing equivalent molar amounts of 264scTCR/IL-2, MART-1scTCR/IL-2 or rhIL-2. Splenocytes were then mixed with NK-sensitive YAC-1 target cells at different ratios to determine cell-mediated cytotoxic activity. Equivalent levels of lytic activity was observed from the mouse splenocytes stimulated by 264scTCR/IL-2, MART-1scTCR/IL-2 or rhIL-2 (Fig. 3A). Short term stimulation of human PBMC or enriched human NK cells with 264scTCR/IL-2 or rhIL-2 also resulted in equivalent levels of cytotoxic activity (Fig. 3B). These results indicate that the scTCR/IL-2 fusion proteins have equivalent bioactivity as rhIL-2 for stimulating cytotoxic immune effector cell activity.

The effect of the scTCR/IL-2 fusion proteins on immune cells was also investigated *in vivo*. Repeated daily intravenous administration of c264scTCR/IL-2 at 32 μg/dose in A375 tumor-bearing nude mice resulted in an increase in serum IFN-γ levels and enlargement of lymph nodes and spleens, consistent with reported effects for rhIL-2 (Rosenstein et al., 1986. J. Immunol. 137:1735). Changes in IL-2R subunit expression were also assessed in immune cells isolated from the spleens, lymph nodes and bone marrow of c264scTCR/IL-2- or MART-1scTCR/IL-2-treated mice.

Splenocytes expressing IL-2Rα (CD25) or IL-2β (CD122) increased approximately three-fold with repeated daily treatments of either scTCR/IL-2 fusion protein such that peak expression of the IL-2R subunits was observed one day after the fourth treatment. In addition, repeated administration of c264scTCR/IL-2 or MART-1scTCR/IL-2 resulted in a treatment-dependent

increase in NK cells with a similar time-course observed for the IL-2R markers. Elevated expression of CD122 and CD25 was also observed in cells isolated from the lymph nodes and bone marrow of mice following repeated daily c264scTCR/IL-2 treatment. These findings are also consistent with the known biological activities of IL-2 and suggest that repeated scTCR/IL-2 fusion protein administration augments further immune responsiveness to the fusion protein via IL-2R-positive cells (Puri et al. 1990 Cancer Res. 50:5543, Depper et al., 1985 PNAS 82:4230; Demaison, 1998 J. Immunol. 161:1977)

Pharmacokinetic profile of 264scTCR/IL-2 fusion proteins in mice and humans

As set forth below, the results of animal and human studies demonstrate that the scTCR/IL-2 fusion protein has a much longer serum half-live and higher serum recovery than rhIL-2. The longer half-life, modest tissue distribution, slow clearance and stable bifunctionality of the scTCR/IL-2 fusion proteins provide significantly more favorable pharmacokinetic properties than are observed for IL-2-based therapeutic agents.

Therapeutic use of rhIL-2 is limited by its very short half life such that patient receiving this drug need to be infused every 8 hours during the FDA-approved 5 day treatment cycle.
The pharmacokinetic parameters of c264scTCR/IL-2, MART-1scTCR/IL-2 and rhIL-2 were evaluated in the HLA-A2.1/K^b-transgenic mouse strain. The presence of the HLA-A2.1 domain, for which c264scTCR/IL-2 is restricted, may influence the pharmacokinetics of this fusion protein and should give a more relevant "humanized" view of the pharmacokinetics than other mouse strains. Mice were injected intravenous with 1.6 mg/kg c264scTCR/IL-2 or MART-1scTCR/IL-2 or 0.4 mg/kg (molar equivalent dose) rhIL-2 and blood was collected at various time points from 5 minutes to two weeks post injection. Serum concentrations of c264scTCR/IL-2 and MART-1scTCR/IL-2 were evaluated using two ELISA formats, one (anti-IL-2 Ab) which detects the intact bi-functional protein and the other (W4F Ab) which detects only the TCR domain. Concentrations of rhIL-2 were detected with a standard IL-2-specific ELISA.

The predicted fit and actual data for c264scTCR/IL-2 and rhIL-2 serum concentrations following the single intravenous bolus injection are shown in Figure 4A. The estimated half-life of 264scTCR/IL-2 using anti-IL2 Ab or W4F Ab ELISAs was 1.82 hrs or 2.00 hrs, respectively. These results indicate that the bi-functional fusion protein was not cleaved *in vivo*. The clearance (Cl) of c264scTCR/IL-2 ranged from 0.376 to 0.493 and the volume of distribution at steady state (Vss) from 0.96 to 1.33 depending on the assay format. The average Vss is about 16% larger than the plasma volume suggesting modest distribution of c264scTCR/IL-2 into tissues. In comparison, rhIL-2 had an absorption half-life ($t_{1/2}$-abs) of ~0.07 hrs and a terminal half-life of 0.39 hrs. The clearance of rhIL-2 was 42.0 ml/hr and the volume of distribution at steady state was 9.93 ml. These results indicate that c264scTCR/IL-2 fusion protein displays a five-fold longer terminal half-life and is cleared >90-fold slower than rhIL-2.

The results comparing the pharmacokinetics of c264scTCR/IL-2, MART-1scTCR/IL-2 and rhIL-2 are shown in Fig 4B. In this study the terminal half-lives of rhIL-2, c264scTCR/IL-2 and MART-1scTCR/IL-2 were determined to be about 0.33, 1.84 and 3.50 h, respectively. Thus, MART-1scTCR/IL-2 has a serum half-life about 11 times longer than rhIL-2. Consistent with the results observed for c264scTCR/IL-2, pharmacokinetics of MART-1scTCR/IL-2 were equivalent whether assessed by the anti-IL-2 or W4F Ab-based ELISAs, indicating that the bi-functional fusion protein was not cleaved apart in vivo.

The results observed in HLA-A2 transgenic mice are borne out by pharmacokinetic analysis for cancer patients treated with fusion protein. The terminal half-life of c264scTCR/IL-2 following a single bolus intravenous infusion (0.015 mg/kg) averaged 4.4 hours (n = 4) and over 75% of the administered dose was detectable in the serum. Again analysis with different ELISA formats verified that the scTCR/IL-2 fusion protein remained intact in the patient's serum. As reported in the product label for rhIL-2 (Proleukin®), studies in humans indicated that upon completion of intravenous infusion, only 30% of the rhIL-2 dose is measurable in the serum. These results are consistent with the very rapid (<1 min) uptake of the majority of rhIL-2 in the lungs, liver, kidney and spleen observed in animal studies. Of the drug remaining in the circulation, the distribution and elimination $t_{1/2}$ for rhIL-2 was 13 and 85 minutes, respectively (Proleukin® Drug Label).

Single-chain TCR/IL-2 fusion proteins exhibit anti-tumor efficacy in vivo

The following study demonstrates that scTCR/IL-2 fusion protein has significantly greater efficacy against well-established human xenograft tumors than rhIL-2 alone.

To determine the therapeutic effects of the scTCR/IL-2 fusion proteins compared to rhIL-2, we examined antitumor activity in a primary tumor growth model with the human A375 cell line in nude mice. Tumor cells were injected subcutaneously into nude mice and tumors were allowed to establish for three days before treatment began. Tumor-bearing mice were injected intravenously with 264scTCR/IL-2 (1.6 mg/kg), the molar equivalent of rhIL-2 (0.4 mg/kg), or the dose volume equivalent of PBS daily for four days, followed by treatment every other day for a total of 9 doses. During the study, tumor growth was measured and the tumor volumes were plotted (Figure 5A). As expected, all mice treated with PBS developed tumors that grew to a large size. All of the mice treated with rhIL-2 developed tumors, while only 50% of mice treated with 264scTCR/IL-2 developed tumors and these remained small throughout the study (Figure 5A). We also examined the effects of 264scTCR/IL-2 on large well-established A375 melanoma tumors. In this study, A375 cells were injected subcutaneously into nude mice and the tumors were allowed to establish and grow to either 75 mm^3 (not shown) or 200 mm^3 (Figure 5B). The mice were then injected intravenously following the same time course as described above with 264scTCR/IL-2 or rhIL-2. Again, treatment with 264scTCR/IL-2 led to marked inhibition of tumor growth and partial to complete regression of tumors in most mice by the completion of the dosing regimen, while tumors in the rhIL-2 group continued to grow at a rapid rate increasing over 4 fold during the course of treatment (Figure 5B).

8. I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: Jan. 7, 2011

Hing Wong

Figure 1. Cell surface residency time of TCR/IL-2 fusions. Cell surface binding of rhIL-2, c264scTCR/IL-2 and MART-1scTCR/IL-2 to IL-2R+ CTLL-2 cells was assessed by flow cytometry. Data points represent normalized level of protein remaining bound to CTLL-2 surface. Curves represent fit of data to a one-phase exponential decay model. Representative results of four independent experiments.

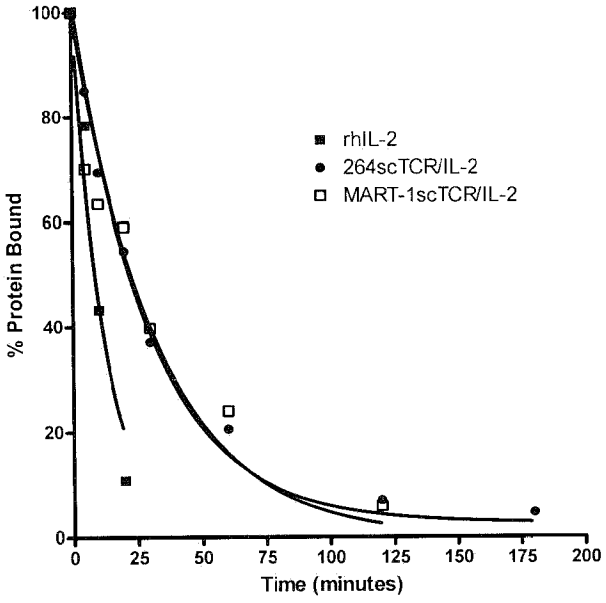


Figure 2. In vitro immune cell proliferative activity of scTCR/IL-2 fusion proteins. CTLL-2 cells were subjected to WST-1 proliferation assays after 2 days of culture with c264scTCR/IL-2, MART-1scTCR/IL-2 or rhIL-2. Each curve represents normalized proliferative responses. Data points are means ± SE of triplicate determinations. EC50 and 95% CI interval were estimated by four-parameter logistic curve fitting analysis.

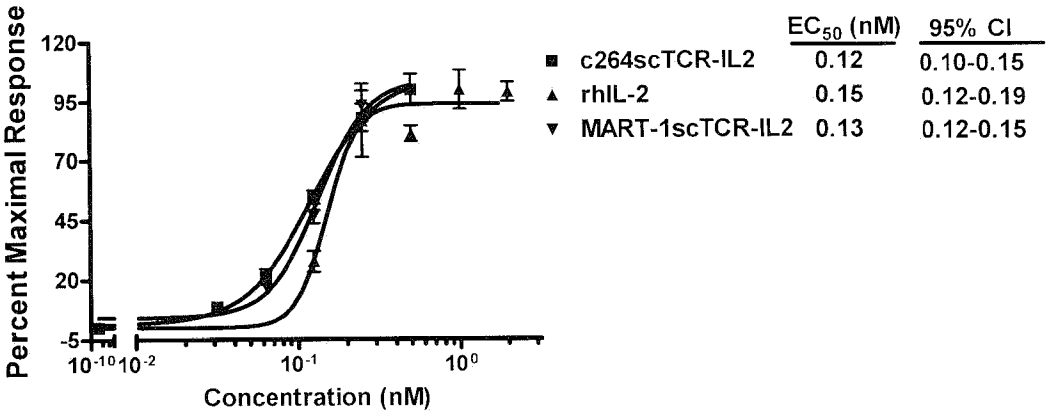
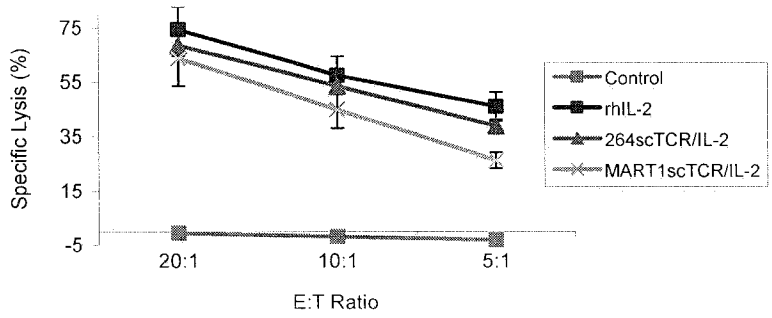


Figure 3. In vitro immune stimulatory activity of scTCR/IL-2 fusion proteins. A) Lytic activity of scTCR/IL-2 fusion protein-activated nude mouse splenocytes was compared to unstimulated (control) or rhIL-2-activated splenocytes in calcein release assays using YAC-1 target cells at different E:T ratios. The specific cytotoxicity was calculated using the following formula: percentage of cytotoxicity = (fluorescent intensity (FI) of cell supernatant of test sample – FI of target cells with medium) ÷ (FI of target cells treated with 0.04% Triton X-100 – FI of target cells with medium) x 100. Representative results of two independent experiments. B) Lytic activity of human PBMCs incubated for 11 hours with 260 IU/mL of 264scTCR/IL-2 or rhIL-2 was compared to unstimulated human PBMCs in calcein release assays using K562 target cells at different E:T ratios.

A)



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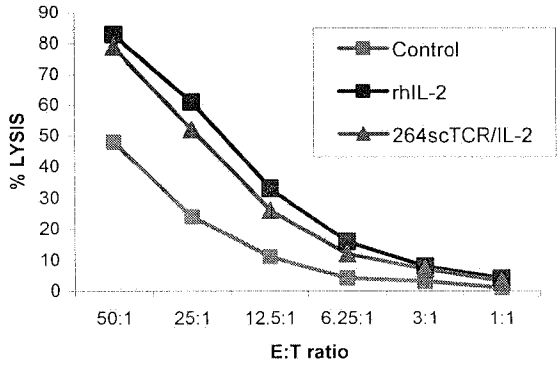
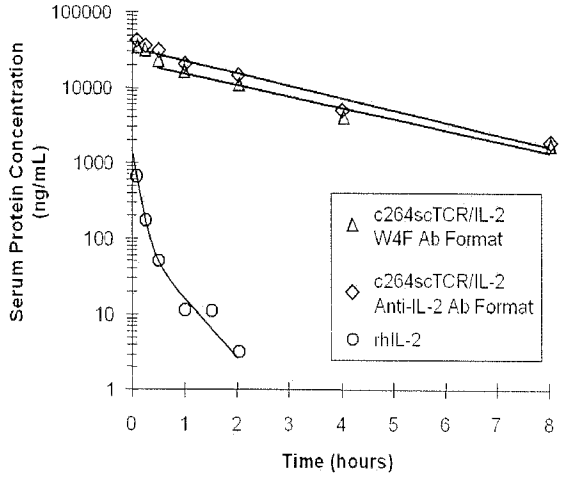


Figure 4. Comparison of the pharmacokinetic profiles of scTCR/IL-2 fusion proteins and rhIL-2. A) c264scTCR/IL-2 or rhIL-2 were administered intravenously to HLA-A2.1/K^b-transgenic mice and serum levels of the proteins were measured by ELISA. The anti-IL2 Ab ELISA measures the concentration of intact c264scTCR/IL-2 molecule whereas the W4F Ab ELISA measures serum concentration of the TCR Cβ domain. The observed concentrations are represented by symbols and the model-fitted curves are represented by lines. B) Comparative pharmacokinetics of MART-1scTCR/IL-2, 264scTCR/IL-2 and rhIL-2 proteins administered intravenously in HLA-A2.1/K^b-transgenic mice.

A)



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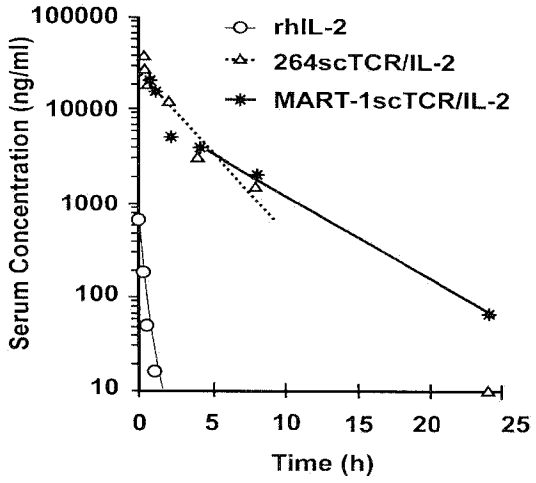
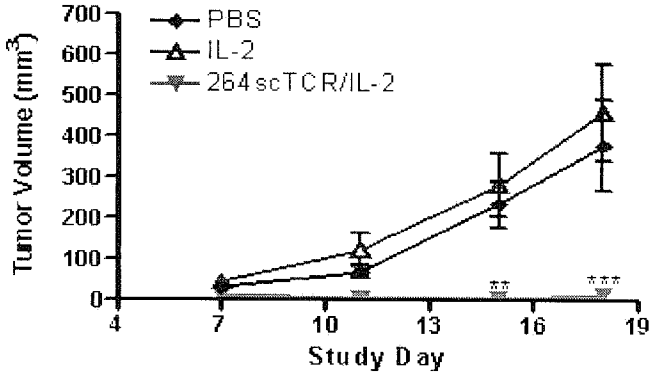


Figure 5. Effect of scTCR/IL-2 on growth of primary tumors in nude mice. A375 tumor cells ($1x10^6$ cells) were injected s.c. into nude mice on study day 1 and tumors were allowed to establish for three days (A), allowed to grow to 200 mm3 (B). Mice were then treated i.v. with 264scTCR/IL-2 (1.6 mg/kg/dose) or rhIL-2 (0.4 mg/kg/dose) daily for 4 days, followed by treatment every other day for a total of 9 doses. Tumors were measured at least twice a week and tumor volumes (mean ± SD) were plotted.

A)



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